perfusing donor organ tissue with Fas ligand; and

introducing [said] the Fas ligand perfused donor organ tissue to said individual.

REMARKS

The 35 U.S.C. §102(e) Rejection

Claim 17 stands rejected under 35 U.S.C. §102(e) as being anticipated by **Bellgrau** et al. This rejection is traversed.

Claim 17 has been amended to recite a specific order in which the donor tissue and the Fas ligand are to be administered, i.e., the donor organ tissue is perfused with Fas ligand first and then the perfused donor organ tissue is introduced to the recipient. In contrast, **Bellgrau** et al. teaches that diabetic rats are injected with islets cells under a renal capsule and that purified Fas ligand is dispensed into the rats through a pump implanted under the renal capsule (see Example 1). Moreover, the pump is programmed to dispense the Fas ligand over a period of time. That is, in **Bellgrau**'s method, the donor organ tissue (i.e., islet cells) and the Fas ligand are

introduced to the recipients through different routes and at different times.

Additionally, the method disclosed in the present invention uses donor tissue that has been engineered to not express Fas ligand. Moreover, the donor tissue provides antigen presenting cells which are capable of eliminating T cells specifically. That is, the present invention uses Fas negative antigen presenting cells. Perfused with the donor tissue, Fas ligand is targeted primarily to the spleen via antigen presenting cells and very seldom to the other sites. This puts Fas ligand in the precise place where it induces a maximum benefit towards interaction with autoreactive T cells, resulting a systemic and not a local tolerance. In contrast, **Bellgrau** et al. teaches Fas ligand therapy in Fas positive cells. In their method, Fas ligand expression by Fas positive cells (i.e., donor tissue) is not protected but toxic and causes the apoptosis of the cells. Moreover, **Bellgrau** et al. does not teach antigen presenting cells or the expression of Fas ligand in the antigen presenting cells. Therefore, **Bellgrau**'s Fas ligand cannot be specifically targeted to the spleen but might migrate to the liver and other tissues and cause high toxicity.

In view of the above remarks, **Bellgrau** et al. does not teach each and every element of Applicants' claim 17 and, in fact, teaches away from the method of claim 17. Accordingly, Applicants request that the rejection of claim 17 under 35 U.S.C. §102(e) be withdrawn.

The 35 U.S.C. §103(a) Rejection

Claims 1, 3-6 and 17 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Süss** et al. Further, claims 1, 3-6 and 17 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Schuler** et al. These rejections are respectfully traversed.

Bellgrau et al. teaches a method for treating T-lymphocyte-mediated primary disease by introducing a cell which is transfected with and expresses a gene encoding Fas ligand. **Bellgrau** et al. differs greatly from the present invention. First, **Bellgrau** et al. does not teach nor suggest antigen presenting cells which express Fas ligand and an antigen. Secondly, **Bellgrau** et al. does not teach or suggest the use of such antigen presenting cells for inducing systemic tolerance to the antigen. Thirdly, **Bellgrau** et al. teaches a

different method of suppressing rejection of a graft, which is discussed under the 35 U.S.C. §102(e) rejection.

Süss et al. teaches that $CD8^+$ dendritic cells express Fas ligand and induces apoptosis of $CD4^+$. In **Süss**'s method, a defined peptide antigen (FERFEIFPK) was added to cultures of CD4 T cells together with $CD8^+$ dendritic cells, resulting in a decreased T cell proliferation (page 1790, col. 1, paragraph 3; page 1791, col. 2, paragraph 2). **Süss** et al. does not teach or suggest a method by using antigen presenting cells expressing Fas ligand and the antigen to induce systemic tolerance to an in-need individual. Neither does **Süss** et al. teach a method of decreasing rejection of a graft by administering Fas ligand perfused donor organ to an in-need individual.

Bellgrau et al. in combination with **Süss** et al. do not provide a person having ordinary skill in this art with the requisite motivation nor expectation of successfully producing Applicants' claimed methods. In fact, there are four major differences between the present invention and the prior art including **Bellgrau** et al. and **Süss** et al.

First, the present invention uses adenovirus AdLoxPFasL combined with the second adenovirus AxCANCre. No prior art

reference describes such a two-virus system as used by the claimed methods. This two-virus system has the advantage of being able to grow at high titers in the 293 cells since neither adenovirus, by themselves, produce high levels of Fas ligand.

Secondly, the claimed method discloses a Fas negative antigen presenting cell line from *lpr/lpr* mice. Previous investigators have used Fas ligand therapy in Fas positive cells. High levels of Fas ligand expression in Fas positive cells result in at least some apoptosis of the cells or even kill the cells. Thus, Fas ligand expression by these cells are not protected but toxic.

Thirdly, the present invention discloses the use of an antigen presenting cell line. The antigen presenting cell line enables loading of this cell line with different antigens that are autoreactive, resulting in the processing of the antigens and specific stimulation of T cells. This includes insulin or GAD65 in the case of insulinitis, mylen basic protein in the case of EAE, MCMV in the case of mouse cytomegalovirus (MCMV) induced chronic post inflammatory disease or adenovirus in the case of prolonging adenovirus infection. This is important since T cells are relatively resistant to Fas ligand-induced apoptosis unless they are stimulated by specific antigen expressed in antigen presenting cells and can also encounter Fas ligand by the

same cell. This provides both the local stimulus to facilitate Fas apoptosis signaling and the production of Fas ligand.

Lastly, the method claimed in the present invention targets Fas ligand primarily to the spleen via antigen presenting cells and very seldom to the other tissues, while previous investigators cannot specifically target Fas ligand since it might migrate to the liver and cause high toxicity. Targeting Fas ligand to the spleen enables the induction of a maximum benefit towards interaction with autoreactive T cells. This produces a systemic and not a local tolerance. The systemic tolerance prevents the immigration of autoreactive cells in the spleen that would migrate to the brain, islet, lung, kidney and other organs. This makes Fas ligand therapy using antigen presenting cells effective in reducing inflammatory response.

In view of the above remarks, the combination of **Bellgrau** et al. and **Süss** et al. do not render claims 1, 3-6 and 17 obvious.

Schuler et al. is a review article and teaches that dendritic cells within thymic medulla are critical in inducing central tolerance (page 320, col. 2). **Schuler** et al. further cites Süss et al. as evidence that $CD8^+$ expressing dendritic cells express Fas ligand at high levels and kill $CD4^+$ T cells. No data are given in **Schuler** et al.

demonstrating the method of inducing antigen-specific systemic tolerance by administering antigen presenting cells expressing Fas ligand and the antigen as claimed in the present invention. As discussed above, **Bellgrau** et al. teaches a different method of immunosuppression. Therefore, the combined teachings of **Bellgrau** et al. and **Schuler** et al. still would not have led to the production of the present invention.

It is the Examiner's opinion that the article by Chen and Wilson (*Nature Biotechnology* 16: 1011-1012, 1998) does not provide evidence that the cited teachings of **Süss** et al. and **Schuler** et al. do not encompass the claimed invention. Applicants disagree.

In their article, Chen and Wilson discuss the "ingenuity" of the present invention. Applicants draw the Examiner's attention that the article was published in 1998, post-dating both the **Süss** et al. (1996) and **Schuler** et al. (1997) references. If the teachings of **Süss** et al. and **Schuler** et al. rendered the present invention obvious, the invention would not have been considered "ingenious" by Chen and Wilson. In fact, since Fas ligand can both protect and damage self tissues, method of disarming the immune system by administering Fas ligand to reduce the T lymphocyte barrier to gene therapy has neither been obvious nor disclosed in the prior art.

Disarming the immune system first requires that the cells producing Fas ligand not be responsive to Fas ligand, as provided in the present invention using antigen presenting cells from *lpr/lpr* mice. Secondly, the autoreactive T cells need to be specifically stimulated for undergoing apoptosis, as done in the present invention by the antigen presenting cells that also produce Fas ligand. Thirdly, the Fas ligand-expressing cells need to move to the site of autoreactive T cells. In the present invention, this site is the spleen and the migration is directed by the Fas ligand-expressing antigen presenting cells. Therefore, Applicants' invention is the first to fulfill the requirements for disarming the immune system by administering Fas ligand. Based on these remarks, the article by Chen and Wilson certainly supports the non-obviousness of the claimed methods, wherein Fas ligand is introduced to and expressed by the antigen presenting cells to induce apoptosis of T cells leading to antigen-specific T-cell tolerance.

In view of the above arguments, Applicants respectfully submit that **Bellgrau** et al. in view of **Süss** et al., and further **Bellgrau** et al. in view of **Schuler** et al. do not render obvious claims 1, 3-6 and 17. Accordingly the rejections of claims 1, 3-6 and 17 under 35 U.S.C. §103(a) should be withdrawn.

Claim 16 stands rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Süss** et al. Further, claim 16 stands rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Schuler** et al. These rejections are respectfully traversed.

Bellgrau et al. teaches a method for suppressing T-lymphocyte-mediated rejection of a graft by introducing into the recipient mammal a cell which expresses the Fas ligand (col. 3, lines 42-45). **Bellgrau** et al. does not teach the use of antigen presenting cells which express Fas ligand and an antigen specific to the graft in their method.

Süss et al. teaches that $CD8^+$ dendritic cells express Fas ligand and induces apoptosis of $CD4^+$ T cells. **Süss** et al. does not teach or suggest a method of creating immune-privileged sites in an in-need individual to decrease the rejection of a graft using Fas-ligand-expressing antigen presenting cells, or specifically dendritic cells. In fact, **Süss** et al. cites prior art for the killing of T cells to create immune-privileged sites by the expression of Fas ligand by Sertoli cells or parenchymal cells (page 1795, left col.). Therefore, **Bellgrau** et al. in combination with **Süss** et al. do not render claim 16 obvious.

Schuler et al. teaches that Fas-ligand-expressing dendritic cells induce tolerance. **Schuler** et al. further cites **Süss** et al. as evidence that $CD8^+$ expressing dendritic cells express Fas ligand at high levels and kill $CD4^+$ T cells. **Schuler** et al. provides no data or even suggestion of a method of creating immune-privileged sites in an in-need individual to decrease the rejection of a graft using Fas-ligand-expressing antigen presenting cells, or specifically dendritic cells. In contrast, the present invention teaches how to produce Fas ligand-expressing cells in an in-need individual vs. demonstrates the normal expression of Fas ligand in $FasL^+$ cells. Lacking the necessary suggestion, the combination of **Bellgrau** et al. and **Schuler** et al. would not have led a person having ordinary skill in this art to the production of the present invention. Moreover, the article by Chen and Wilson, as discussed above, provides a strong support that the present invention, wherein Fas ligand is introduced to and expressed by the antigen presenting cells to create immune-privileged sites so as to prevent rejection of the graft, is free of prior art.

Based on the above remarks, **Bellgrau** et al. in view of **Süss** et al. or in view of **Schuler** et al. do not render claim 16

obvious. Accordingly, Applicants respectfully request that the rejection of claim 16 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Office Action mailed April 21, 1999. Applicants submit that the pending claims are in condition for allowance. If any issues remain, please telephone the attorney of record for immediate resolution.

Respectfully submitted,

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